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Laminin-8/9 is synthesized by rat glomerular mesangial cells and is required for PDGF-induced mesangial cell migration

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Laminin-8/9 is synthesized by rat glomerular mesangial cells and is required for PDGF-induced mesangial cell migration.

Background. Laminin (LM), the major glycoprotein component of basement membranes is expressed as multiple isoforms in a developmentally regulated and tissue-specific manner. LM α4 has a limited tissue distribution and is highly expressed in the developing glomerulus. In the present study, we investigate the in vivo and in vitro expression and function of LM α4 in the glomerulus.

Methods. LM α4 expression was examined by Northern blot, reverse transcription polymerase chain reaction (RT-PCR), Western blot, and immunofluorescence microscopy. Mesangial cells (MC) were plated on purified LM-1, LM-2, and LM-8/9. Immunofluorescence microscopy was performed to examine the cellular phenotypes induced by LM-1 and LM-8/9. A modified Boyden chamber method was used to assess laminin participation in platelet-derived growth factor (PDGF)-stimulated migration.

Results. mRNA for LMα4 is expressed in cultured rat MC, and isolated rat and mouse glomeruli, but not in cultured rat glomerular epithelial cells or glomerular endothelial cells. Using antibodies specific for LM α4, a 240 kD band was detected in MC extract and a slightly smaller band was identified in extracted rat glomeruli. Purified LM-8/9 had MC adhesive activity comparable to LM-1 and LM-2. MC attached to LM-8/9 exhibited a unique phenotype. In contrast to LM-1, attachment of MC to LM-8/9 produced a highly arborized cell morphology with significantly reduced formation of focal contacts or stress fibers. LM α4 is utilized by MC during PDGF-stimulated migration.

Conclusion. LM α4 is synthesized by MC and persists in the mature glomerulus. LM-8/9 stimulates a unique cellular morphology, and they are utilized in PDGF-induced migration. These factors suggest that LM α4 plays an important role in MC differentiation and in the maintenance of MC phenotype.

The kidney expresses a variety of laminin (LM) isoforms that are regionally restricted and that contribute to renal development. Several recent publications summarize what is known about laminin isoform expression in adult kidneys and during nephrogenesis [1–5]. As glomerular development in mice progresses, LM α5 becomes the prominent α chain in the glomerular basement membrane (GBM), where it replaces the LM α1 chain. LM α4 is initially expressed in the developing mesangium, which is followed by expression of LM α2. A transition in β chains from LM β1 to LM β2 also occurs in the glomerulus during the capillary loop stage. Disruption of LM α1, α5, or β2 expression results in the loss of normal function or arrested development [6–8]. LM α1 plays a role in maintaining epithelial cell polarity and is required for kidney tubulogenesis [4, 9]. The lack of LM α5 expression results in the absence of vascularized glomeruli [7]. In the LM β2 null-mutant, the glomerulus appears normal at birth, but soon after, the mutants develop massive proteinuria and effacement of foot processes [8]. These studies confirm the importance of regionally specific laminin isoform expression to kidney development.

LM α4 is a truncated chain similar to LM α3A and has been shown to associate with LM β1, β2, and γ1 to form LM-8 (α4β1γ1) and LM-9 (α4β2γ1). LM α4 is highly expressed during development. In adult tissues LM α4 is found in the basement membranes of lung alveolar septa, capillary, and larger vessel walls and cardiac and smooth muscle fibers [1, 10, 11]. Recent studies have identified LM-8 as an adhesion and migratory substrate for a variety of cell types [12–14]. LM α4 deficiency results in transient neonatal hemorrhages, impaired angiogenesis, and improper localization of motorneuron synapses [15, 16]. The lack of LM α4 expression results in delayed deposition of collagen IV and nidogen into the capillary basement membrane. Yet, the specific biologic functions and complete localization of LM α4 remain incomplete.

In the present study, we investigated the in vivo and in vitro expression and function of LM α4 in the glomerulus. Cultured MC express LM α4, mRNA, and protein, whereas glomerular, visceral, epithelial, and endothelial cells do not. LM α4 persists in the mature rat glomerulus

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in a mesangial distribution. LM-8/9 supports MC adhesion and promotes a unique phenotype. In addition, binding to LM α4 is required for platelet-derived growth factor (PDGF)-stimulated MC migration.

METHODS

Reagents and antibodies

Engelbreth-Holm-Swarm sarcoma (EHS)-laminin (α1β1γ1, laminin 1, LM-1) and anti-collagen IV antibodies were obtained from Collaborative Research, Inc. (Waltham, MA, USA). Human merosin was purchased from Life Technologies (Rockville, MD, USA). Polyclonal anti-LM-1, anti-fibronectin (FN), anti-LM β1, and anti-LM β2 antibodies were produced in our laboratory as previously described [17, 18]. Antibodies to talin, vinculin, and vimentin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). A goat antibody to a peptide from the carboxy terminus of LMα4 (V-20) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Oregon green™ 514 phalloidin and Cy3-conjugated anti-mouse IgG antibodies were purchased from Molecular Probes (Eugene, OR, USA). Biotinylated and fluorescein-conjugated, species-specific anti-immunoglobulin (Ig) G antibodies were purchased from Jackson Laboratories (West Grove, PA, USA). Horseradish peroxidase (HRP)-conjugated anti-mouse IgG was purchased from Pierce (Rockford, IL, USA). PDGF-BB was purchased from Upstate Biotechnology (Lake Placid, NY, USA).

Polyclonal rabbit antibodies to LM α2 and α4 were generated as follows. A LM α4 fusion protein containing nucleotides 4392-5201 of mouse laminin α4 [11] was cloned in frame into the pESP-2 vector (Stratagene, La Jolla, CA, USA) using the manufacturer's instructions. The glutathione-S-transferase (GST) fusion protein was expressed in the yeast *Schizosaccharomyces pombe* strain, SP-Q01. Following cell lysis in phosphate-buffered saline (PBS) (140 μm NaCl, 2.7 μm KCl, 10 μm Na_2HPO_4, 1.8 μm KH_2PO_4) containing 1 μm ethylenediaminetetraacetic acid (EDTA), 1.5% N-lauryl-sarcosine, and 3% Triton X-100, the fusion protein was purified by affinity chromatography. This material was used to produce polyclonal antibodies designated anti-α4G. Primary immunization was administered intramuscularly with 60 μg affinity-purified GST-LMα4 fusion protein suspended in complete Freund's adjuvant (CFA). Booster immunizations were administered subcutaneously at two-week intervals using 30 μg antigen in incomplete Freund's adjuvant. These antibodies were characterized by Western blot and reacted with protein bands similar to those identified with rabbit anti-LM α4 (provided by Dr. Jeffery Miner, Washington University, St. Louis, MO, USA) [1] and goat antibody to a peptide from the carboxy terminus of LMα4 (V-20). For production of anti-α2G antibodies, a portion of the globular domain was produced by reverse transcription polymerase chain reaction (RT-PCR) from rat whole kidney RNA using the following primers: 5′ primer CTAAGTTGCCACCAAT GTCCGAGG, 3′ primer GGATCTTTCACCACAGC GTTCGTT. The resulting product was sequenced and confirmed to represent LM α2. It was inserted in frame into the mammalian expression vector pCDNA3.1 (Invitrogen, Carlsbad, CA, USA). Immunization was accomplished by intramuscular injection of purified plasmid as previously described [19, 20]. Primary immunization and monthly booster immunizations were performed with 1 mg plasmid diluted in PBS. These antibodies were characterized by Western blot and reacted with protein bands of similar molecular weights as compared to anti-LMα2 antibodies (obtained from Dr. Kevin Campbell, University of Iowa, Iowa City, IA, USA) [21].

Cell culture

Rat aortic vascular smooth muscle cells (VSMC) were a gift from Dr. Marc Mayberg (Veterans Affairs Puget Sound Health Care System, Seattle, WA, USA) [22]. Rat MC were established from primary glomerular explants and characterized as previously described [23–25]. MC were propagated in standard medium of RPMI 1640 (BioWhittacker Bioproducts, Walkersville, MD, USA) supplemented with 20% fetal calf serum (FCS) (Hyclone Laboratories, Inc., Logan, UT, USA), transferrin, selenous acid, glutamine, penicillin, and streptomycin [23, 26] and studied at passages 8-9. Rat glomerular visceral epithelial cells and endothelial cells were characterized and cultured as previously described [27].

Attachment assay

Twenty-four–well plates (Corning, Corning, NY, USA) were coated by incubating with proteins diluted in PBS (5 to 20 μg/well) overnight at 4°C, followed by treatment with 3% bovine serum albumin (BSA) for 2 hours at room temperature to block the remaining protein-binding sites. Following trypsinization, MC were plated at 50,000 cells/well in serum free media [Dulbecco's modified Eagle's medium (DMEM), 1% BSA, transferrin, selenous acid, glutamine, penicillin, and streptomycin] and incubated at 37°C for 2 hours. This incubation period allowed MC to attach and spread on the coated matrix, but did not allow sufficient time for expression of MC-synthesized laminin. Unattached cells were removed by washing with PBS and the remaining cells were fixed in 2% paraformaldehyde in PBS and stained with 0.5% crystal violet in 20% methanol (MeOH). Attached cells were counted manually under the microscope at ×400 magnification. Each condition was performed in triplicate and the mean of 3 fields per condition in two separate experiments was determined.

Migration assay

Migration of MC was assayed by a modified Boyden chamber method using polycarbonate membranes with 8 μm pore sizes (Nalge Nunc, Naperville, IL, USA) in a 24-well tissue culture plate [28, 29]. MC were trypsinized and plated in the upper chamber at 50,000 cells per well in RPMI 1640 containing 2% FCS. Medium containing 50 ng/mL PDGF-BB was placed in the lower chamber. In some experiments, heat-inactivated normal rabbit serum, anti-LM-1, anti-α2G, or anti-α4G serum was added to the medium in the upper chamber at a concentration of 1% to assess the effect on migration. The chambers were incubated at 37°C for 7 hours. The time course of this assay allows MC to synthesize and assemble extracellular matrix proteins, including laminin. After incubation, the filters were washed with PBS, fixed with 2% paraformaldehyde in PBS and stained with 0.5% crystal violet in 20% MeOH. Migrated cells on the lower surface were counted under the microscope at ×400 magnification. The mean of 4 fields per condition in three separate experiments was determined.

Immunofluorescence microscopy

Normal adult rat kidney was snap-frozen in precooled isopentane and cryostat sectioned at 4 μm. Cultured MC were grown in four chambered slides (Nalge Nunc) and fixed with 2% paraformaldehyde in PBS for 20 minutes at room temperature. Samples were stained by indirect immunofluorescence microscopy by routine methods [17]. Slides were incubated with LM α4 antibodies or control serum overnight at 4°C, washed three times with PBS, and incubated with fluorochrome-conjugated secondary antibody. Controls included treatment with preimmune serum from each of the species represented in the primary antibodies, as well as direct staining with each of the fluorochrome conjugates used. For some experiments four chamber glass slides were coated by incubating with proteins diluted in PBS (5 to 20 μg/well) overnight at 4°C, followed by treatment with 3% BSA for 2 hours at room temperature to block the remaining protein-binding sites. MC were plated in standard medium containing 1% FCS and incubated at 37°C for six hours. Unattached cells were removed by washing with PBS and the remaining cells were fixed in 2% paraformaldehyde in PBS. Prior to staining, the slides were blocked for 2 hours at room temperature with 5% heat inactivated fetal bovine serum (FBS) and 1% BSA in PBS.

Northern blot and PCR analysis

Glomeruli from adult male Sprague Dawley rats and C57B1/6J mice were isolated by differential sieving [30]. RNA was extracted from MC, glomerular epithelial cells (GEC), glomerular endothelial cells (GEnC), isolated rat and mouse glomeruli, rat whole kidney, and rat heart by routine methods [18]. Twenty μg per lane of total RNA was separated by electrophoresis on a 1% agarose denaturing gel, transferred to nitrocellulose, and fixed with a Stratagene Stratalinker (Stratagene) as described previously [18]. Blots were hybridized with probes to LM α4 (NA 4619-5608) [11], and 28s rRNA. Prior to amplification RNA was Dnase-treated using DNA-free™ (Ambion, Austin, TX, USA) following the manufacturer's instructions. The following primers were used for amplification LM α4, GGTCAGGTGACTCGCTTTG (NA 3575-3594) and GCTCTTAACGTGCCGTCTGT (NA 3829-3848) [11]. Primers were used under the following conditions: 95°C for 2 minutes, followed by 35 cycles at 95°C for 0.5 minutes, 55°C for 1 minute, and 72°C for 1 minute, followed by 72°C for 7 minutes. The identity of the RT-PCR products was confirmed by Dyedeoxy sequencing (Applied Biosystems, Foster City, CA, USA).

Purification of laminin-8/9

Laminin was purified from MC grown to superconfluence. The cell layer was initially extracted with an EDTA extraction buffer [150 mM NaCl, 50 mM Tris-HCL, 10 mM EDTA, 5 mM N-ethylmaleimide, and 5 mM phenylmethylsulfonyl fluoride (PMSF)] and subjected to ion exchange chromatography with DEAE-Sepharose Fast Flow (Amersham Biosciences, Newark, NJ, USA) as described by Lindblom et al [31]. The laminin-containing peak was dialyzed in heparin buffer A (10 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, 0.5 mM N-ethylmaleimide) and applied to a 5 mL heparin HiTrap affinity column (Amersham Biosciences) equilibrated with buffer A. The column was washed with buffer B (10 mM Tris-HCl, 300 mM NaCl, 2 mM EDTA, 0.5 mM N-ethylmaleimide) and laminin-8/9 eluted with buffer D (10 mM Tris-HCl, 500 mM NaCl, 2 mM EDTA, 0.5 mM N-ethylmaleimide) [32]. Purity was confirmed by sodium dodecyl sulphate protein agarose gel electrophoresis (SDS-PAGE) Western blot analysis.

Western blot

Extracts of whole glomerular matrix (WGM) were prepared from isolated rat glomeruli by sonicating the samples prior to an overnight incubation in PBS containing 0.05% Triton X-100, 0.025 M EDTA, 0.1 mol N-ethylmaleimide, 2 μM PMSF and 0.02 mg/mL pepstatin. Samples of cultured cell layers and heart extract were prepared as previously described [18]. Electrophoresis was performed under reducing conditions in 5% SDS-polyacrylamide gel containing urea, followed by transfer to nitrocellulose (Schleicher and Schuell, Keene, NH, USA) [33]. Immunoblotting was performed as described previously [18].

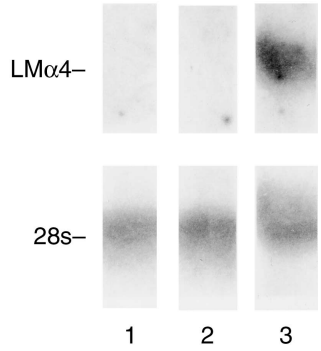

Fig. 1. Northern blot of laminin α4 expression in glomerular cells. The 6.5 kb message for LM α4 is present in MC (lane 3), but not GEC (lane 1) or GEnC (lane 2).

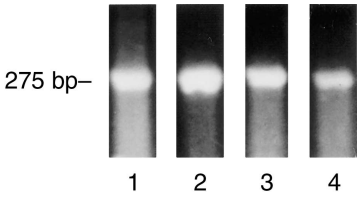

Fig. 2. Reverse transcription polymerase chain reaction (RT-PCR) for LM α4. Product for LM α4 is present in samples from rat heart (lane 1), which served as a positive control, cultured rat MC (lane 2), isolated glomeruli from adult rat and mouse (lanes 3 and 4).

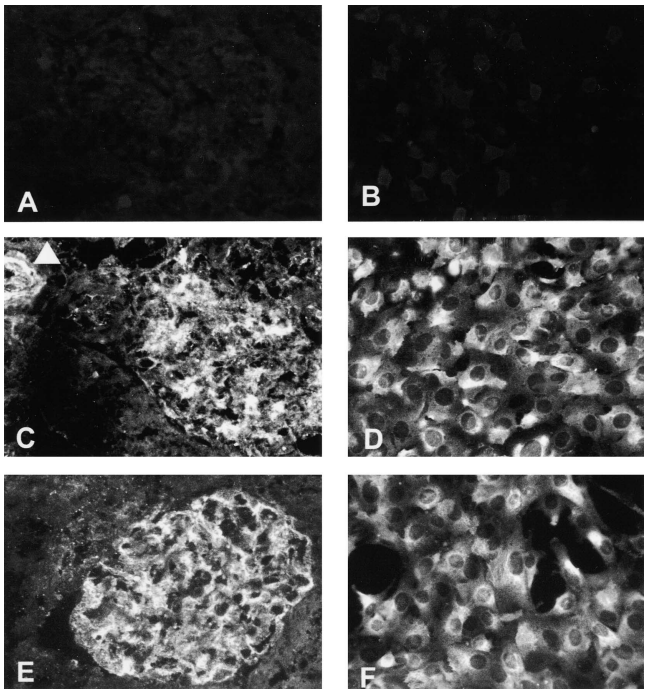

Fig. 3. Immunofluorescence microscopy. (*A*, *C*, *E*) Normal rat kidney and cultured rat MC (*B*, *D*, *F*) treated with preimmune rabbit serum (A, B), anti-LMα4G antibodies (C, D), or goat anti-LMα4 peptide (V20) (E, F). Both the mesangial matrix and the vessel wall (arrowhead) are stained by anti-LMα4G. In cultured cells, LM α4 exhibits a cytoplasmic pattern consistent with synthesis. Original magnification, ×400.

RESULTS

Expression of LM α4

Previous studies of LM α4 expression in the mouse have shown very strong expression during development that diminishes as the glomerulus reaches maturity [1]. More recent studies have demonstrated a persistence of LM α4 in the adult mouse glomerulus [34], as well as in adult human glomeruli [35]. To evaluate mRNA expression of LM α4 in the rat, we performed Northern blot analysis using RNA from cultured rat GEC, GEnC, and MC. Strong expression of LM α4 was detected in MC, but not in GEC or GEnC (Fig. 1). To correlate these data with in vivo expression, RNA from isolated adult rat and mouse glomeruli were evaluated by RT-PCR. As shown in Figure 2, a product of approximately 275 bp was detected in cultured rat MC and whole glomerular RNA from both rat and mouse. RNA from rat heart served as a positive control. Sequencing confirmed that the PCR products represented LM α4 (data not shown).

Additional studies were performed to confirm the synthesis of LM α4 protein. Anti-α4G antibodies and the commercial goat anti-LMα4 peptide were used to stain normal rat kidney, as well as cultured rat MC. LM α4 was detected in the mesangium and in the arteriolar vessel walls using anti-α4G antibodies and the anti-LMα4 peptide (Fig. 3). Similar staining was observed in sections of mouse kidney (data not shown). In addition, staining of cultured MC with both anti-LMα4 antibodies showed granular staining consistent with intracellular synthesis (Fig. 3). When cells grow beyond confluency and deposit a fibrillar extracellular matrix, laminin and staining for LMα4 concentrate in areas of cell-cell contact (not shown). Preimmune serum shows no nonspecific staining under the conditions of these experiments. To confirm the specificity of anti-α4G antibodies Western blot studies were preformed. Matrix extracted from cultured rat MC, as well as isolated rat glomeruli and heart were examined. Anti-α4G, but not normal rabbit serum, stains a pair of bands at 240 to 230 kD in MC matrix, and a slightly smaller band in glomerular matrix and heart (Fig. 4). As the arteriolar vessel walls stained prominently in normal rat kidney, we analyzed the matrix extract from rat vascular smooth muscle cells (VSMC) by Western blot. Anti-α4G antibodies identify bands in VSMC similar to those present in MC extracts. Anti-α4G does not react with LM-1, LM-2 (Fig. 4), or with matrix extracted from GEC or GEnC (data not shown). The apparent size of 210 to 240 kD is consistent with previous reports that identify LM α4 as a 180 to 240 kD protein [1, 10, 36, 37]. Based on these data, LM α4 is expressed by cultured MC, but not GEC or GEnC.

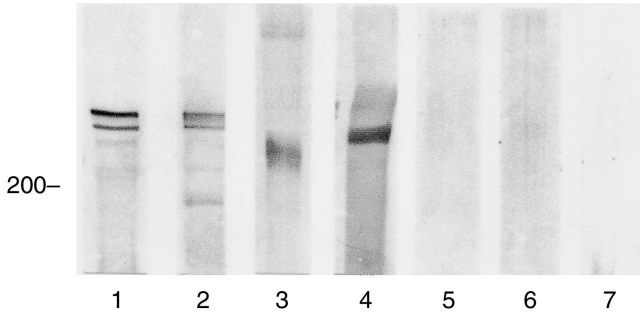

Fig. 4. Western blot for LM α4. Anti-α4G detects a pair of bands at 240 and 230 kD in matrix extracted from cultured rat MC and VSMC (lanes 1 and 2). A slightly smaller band at 220 kD is detected in adult rat whole glomerular matrix (WGM) (lane 3) and heart (lane 4). Anti-α4G does not react with LM-1 or LM-2 (lanes 5 and 6, respectively). Lane 7 shows normal rabbit serum control.

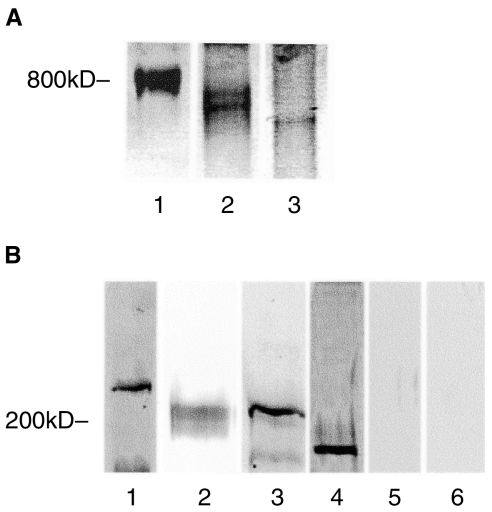

Fig. 5. Purified LM-8/9. (*A*) Silver stain. LM-1 (lane 1), LM-2 (lane 2), and LM-8/9 (lane 3) were electrophoresed under nonreducing condition using a 3.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel. (*B*) Western blot. Purified LM-8/9 was electrophoresed under reducing condition using a 5% SDS-PAGE gel and transferred to nitrocellulose. Strips were probed with anti-α4G (lane 1), anti-LM-1 (lane 2), anti-LM β1 (lane 3), anti-LM β2 (lane 4), anti-fibronectin (FN) (lane 5), and anti-collagen IV (lane 6).

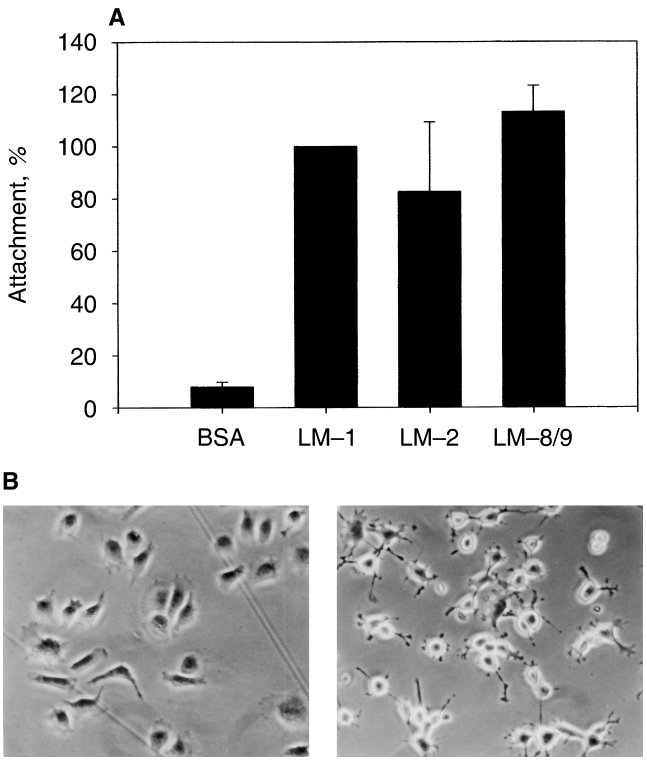

Fig. 6. MC adhesion to LM-8/9. (*A*) MC were plated in serum-free media in 24-well plates coated with bovine serum albumin (BSA), laminin-1, laminin-2, or laminin-8/9. Bars represent the mean and standard deviation of duplicate experiments performed in triplicate. Adhesion is shown as percent of LM-1 designated as 100%. (*B*) Light micrographs of MC plated on LM-1 or LM-8/9. Original magnification, ×100.

This expression of LM α4 is shared by VSMC. In addition, LM α4 mRNA and protein persist in the mature glomerulus in both rats and mice.

The relationship of laminin α4 to MC phenotype and function

To further study the effects of LM α4 on MC, laminin was purified from the cell layer of cultured MC by ion exchange and heparin affinity chromatography. As shown in Figure 5A, MC laminin migrates as a single ~600 to 650 kD band ahead of LM-1 and LM-2 (~800 kD and 700 kD, respectively) under nonreducing conditions. This size is consistent with previous reports for LM α4 containing isoforms [1, 12, 13]. Using chain specific antibodies the purified material contains LM α4, β1, and β2. Anti-LM-1 antibodies identify a band consistent with the LM β1/γ1 chains, but LM α1 was not detected. In addition, no collagen IV was detected and only a minor amount of fibronectin (Fig. 5B). These data show that MC laminin contains LM-8/9.

The cell adhesive properties of LM-8/9 were compared to those of LM-1 and LM-2. As shown in Figure 6A, MC plated in serum free medium attached equally well to LM-8/9 as to the other test substances; however, the overall cell morphology induced by LM-8/9 was strikingly different. MC attachment to LM-8/9 resulted in a condensed cell body with long, thin armlike processes with some lamellae-like areas, whereas attachment to LM-1 resulted in a spread cell with more regular cell margins (Fig. 6B). To better visualize the cell morphology and the underlying cytoskeleton, MC were plated on LM-1 or LM-8/9 coated glass slides and stained with phalloidin, vimentin, vinculin, and talin. MC plated on LM-1 were broadly spread with long actin filaments that crisscrossed the cell (Fig. 7). Vimentin formed a perinuclear network and was present in microspikes at the cell periphery (Fig. 7). In addition, prominent clusters of

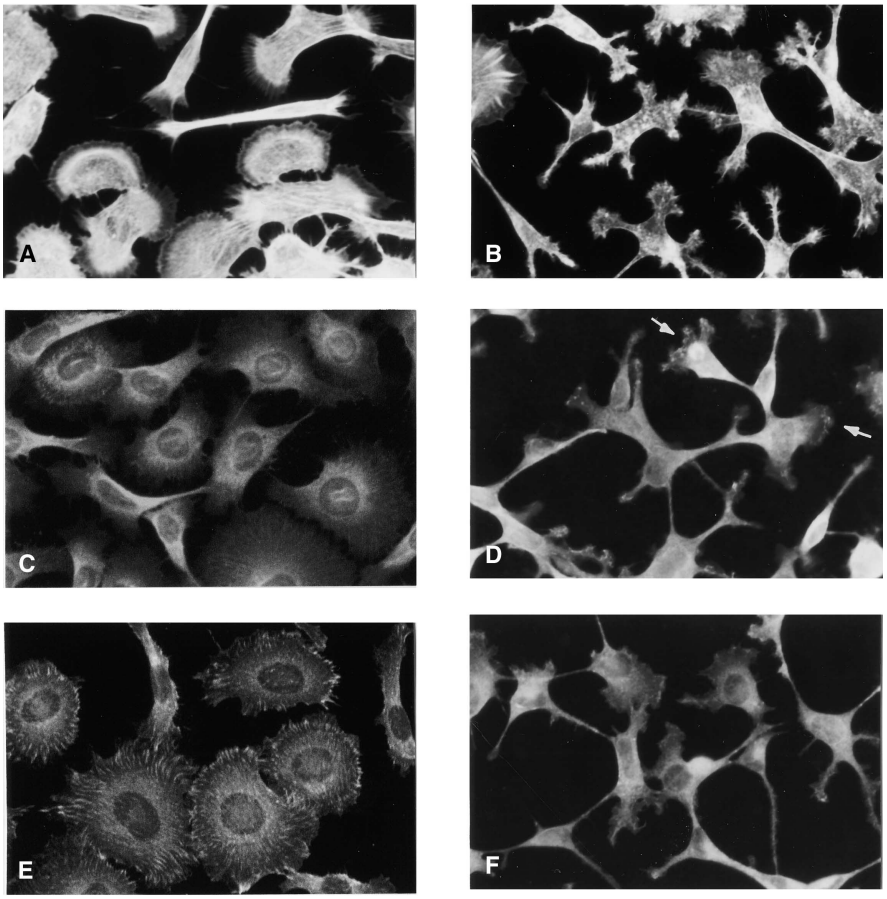

Fig. 7. Immunofluorescence microscopy of laminin-induced cellular phenotype. (*A*, *C*, *E*) MC were plated on glass slides coated with LM-1 (*B*, *D F*) or LM-8/9 and stained with phalloidin (A, B), vimentin (C, D), and vinculin (E, F). Note that LM-8/9 induced an irregular network of short actin fibers (B) with relocation of vimentin to the cell periphery (arrows) (D) and significantly reduced vinculin-positive clusters (F) as compared to LM-1. Original magnification, ×400.

vinculin aligned with actin fibers within the cell body, as well as at the periphery of the cell perpendicular to the cell membrane in a focal adhesion-like pattern (Fig. 7). Talin colocalized with vinculin, resulting in the same pattern of staining (data not shown). These staining patterns suggest a broadly spread cell with edges that are tacked down by focal adhesions. In contrast to LM-1, LM-8/9 was associated with extension of actin filaments into long filopodia with disassembled actin in the central part of the cell (Fig. 7). In addition to the perinuclear network, vimentin was also expressed at the cell periphery in the ends of larger filopodial-like areas (Fig. 7). Distinct staining for vinculin (Fig. 7) and talin (not shown) was also present at the ends of the filopodial extensions, indicating that MC plated on LM-8/9 have a limited number of focal adhesions. Therefore, MC adhesion to LM-8/9 results in a different arrangement of the cytoskeleton, affecting both actin fibers and the intermediate filament, vimentin, as well as significantly reducing the formation of vinculin- and talin-containing focal adhesions. The reduced number of focal adhesions is consistent with the more migratory response described above and shown below in response to PDGF.

The phenotype described above for MC plated on LM-1 indicates that they are attached and spread with well-developed stress fibers and focal adhesions. The reduction of stress fiber and focal contact formation that we observed in MC plated on LM-8/9 has also been demonstrated in cells plated on LM-10/11 [38]. Unlike LM-1 and LM-2, LM-10/11 and LM α4-containing isoforms are potent migratory substrates for a variety of cell types [12–14, 38]; thus, the reduction in focal adhesion formation may help facilitate migration on these laminin isoforms. To examine whether LM α4 has a functional role in MC migration we used a modified Boyden chamber, PDGF-stimulated migration assay. In previous studies, MC have been shown to migrate through a porous membrane in a dose-dependent manner toward increasing concentrations of PDGF [28]. As shown in Figure 8, MC migration in response to treatment with 50 ng/mL PDGF-BB resulted in a 2.5-fold increase over untreated controls. The addition of anti-LM α4G antibodies to the migration chamber completely blocked the increase in migration induced by PDGF, whereas normal rabbit serum and anti-α2G antibodies had no affect. Antibodies to LM-1 partially inhibited PDGF-mediated MC migration. As previously reported, MC do not express LM α1; therefore, LM-1 antibodies react with only LM β1 and

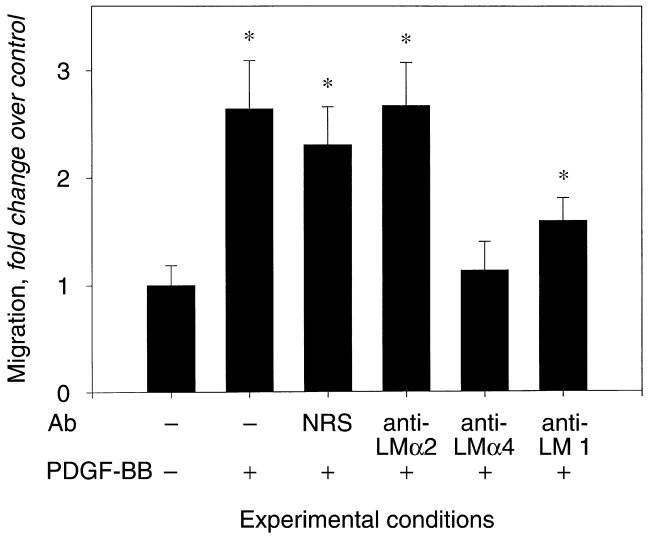

Fig. 8. PDGF-stimulated MC migration. MC were treated with PDGF with and without the addition of anti-α4G, anti-LM-1, anti-α2G antibodies or normal rabbit serum. PDGF caused a 2.5-fold increase in migration as compared to untreated control. The addition of anti-LMα4G and LM-1 antibodies blocked the increase caused by PDGF. Normal rabbit serum and anti-α2G antibodies had no effect on PDGF-stimulated migration. Analysis of variance (ANOVA) was performed. $*P < 0.05$.

γ1 produced by cultured rat MC [18]. Binding of anti-LM-1 antibodies to LM-β1/γ1 chains may partially limit attachment to LM-8/9. These data suggest that MC predominately utilize the LM α4 globular domain of LM-8/9 during PDGF-stimulated migration.

DISCUSSION

The results of this study show that cultured rat glomerular MC synthesize LM α4, whereas rat GEC and GEnC do not. Previous immunohistochemical studies have shown high levels of LM α4 expression in the developing mouse glomerulus, with increased staining for other laminin α chains in the mature glomerulus [1]. By the use of multiple techniques, we have demonstrated in vivo expression of LM α4 mRNA and protein in isolated glomeruli from both adult rats and mice. Although the expression of LM α4 is reduced in the mature glomerulus as compared to its developmental expression, it is retained in a mesangial matrix pattern. Glomerular staining of LM α4 has also been demonstrated in adult mouse [34] and human kidney [35]. Like MC, the related VSMC also expresses the LM α4 chain in culture and in vivo. Because LM α4 is also expressed by smooth muscle cells of the intestine [39], it may play a role in maintaining the phenotype and function of smooth muscle–type cells.

In cultured MC, LM α4 is complexed with LM β1 or β2, and γ1 showing that MC express LM-8 and LM-9. The LM α4 chain isolated from cultured MC had an apparent molecular weight of 240 kD. These data show that the 240 kD laminin α chain previously found in immunoprecipitates of biosynthetically labeled proteins is LMα4 [3, 18, 25]. The LM α4 protein identified in rat glomeruli and heart was slightly smaller at approximately 210 kD. Other studies have shown similar variability in the size of LM α4 extracted from tissues [1, 10, 36, 37]. Studies with recombinant LM α4 fragments have shown proteolytic processing within the globular domain [40]. Cleavage can occur at three different polypeptide bonds, thereby resulting in the generation of molecules of slightly different sizes, as well as loss of tissue incorporation of the α4LG4-5 fragment. In addition, the N-terminal domain of LM α4 can be modified by chondroitin sulfate [34]. Recently, alternative splice products of LM α4 have been shown to occur in human tissues [41]. Thus, the differences in molecular weight for LM α4 derived from cultured cells or tissue extracts may be due to differences in proteolytic processing or other pre- or posttranslational modifications. These modifications may account for unique tissue specific variations in the LM α4 polypeptide and its function, as well as the variable detection of LM α4 in the glomerulus when antibodies recognizing different epitopes are used.

One major function of laminin is to support cellular adhesion through a variety of integrin and non-integrin receptors that are involved in the transfer of signals between the extracellular matrix and the cell interior. Tissue specific expression of laminin isoforms may influence cellular behavior by the activation and clustering of distinct sets of receptors and focal-adhesion linker proteins. Laminin-8 (α4β1γ1) promotes cell adhesion in a number of cell types [13, 14, 42]. In the present study LM-8/9 was shown to support MC adhesion and to promote a unique highly arborized cellular phenotype. This phenotype lacks the stress fiber formation of the actin cytoskeleton and redistributes the intermediate filament vimentin as compared to cells plated on LM-1. A LM α4 and vimentin-containing adhesion structure has recently been described in microvascular endothelial cells [43]. Although further study is needed, these data suggest that MC interacting with LM α4 may also form this unusual vimentin-associated adhesion. Vinculin and talin are F-actin binding proteins that are commonly recruited into focal adhesion complexes [44]. Although prominent clustering of these proteins into focal adhesions was seen in MC plated on LM-1, attachment to LM-8/9 resulted in a significant decrease in the apparent number of focal adhesions. As both the cytoskeleton and focal adhesion complexes play crucial roles in signal transduction, the different arrangement of molecules described above may be linked to the transmission of distinct signals. Furthermore, the variation in the number of adhesions may contribute to the reported differences in facility of migration of cells plated on different laminin isoforms [12, 14, 38, 43, 45, 46].

During nephrogenesis, MC progenitors that are presumed to be derived from the metanephric mesenchyme [47, 48] cluster in the region of the mesenchymal condensates as the glomerulus begins to develop. MC migrate into the vascular cleft, proliferate, and differentiate into mature MC. MC development requires PDGF, as mice deficient for PDGF B-chain or the PDGF-β receptor fail to develop a glomerular mesangium [49]. PDGF is a potent mitogen for MC, and it stimulates migration [48, 50]. Because there is increased expression of LM α4 by MC during glomerulogenesis and PDGF is known to stimulate MC migration, we examined the role of LM-8/9 in PDGF-induced MC migration. As antibodies to the globular domain of LM α4 completely block PDGF-stimulated increase in MC migration, it suggests that MC binding to this region of LM α4 is required. In previous studies, we have shown that cultured rat MC synthesize LM β1 and γ1, but not α1; thus, partial blockade of PDGF-stimulated MC migration with antibody to LM-1 suggests that binding to the β1 and γ1 chains of laminin-8 may interfere with MC adherence to LM-8. Thus, LM α4-containing isoforms play a critical role in MC migration and the associated phenotype.

CONCLUSION

These studies show that LM-8/9 are produced by MC in culture and expressed in the glomerulus in vivo. This LM isoform induces a unique phenotype characterized by few focal adhesions, which might facilitate migration on this substrate. Because PDGF-induced MC migration can be blocked by antibodies that disrupt cell attachment to the globular domain of the LM α4 subunit, it supports a critical role for this LM isoform in MC migration during glomerulogenesis and in disease states associated with increased PDGF expression.

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